*INQUILINIC PROTOZOA FROM FRESHWATER GASTROPODS. I. *TRICHODINA HELISODURIA* N. SP. FROM *HELISOMA DURYI* SAY, IN FLORIDA

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Since Ehrenberg (1838) recorded *Trichodina pediculus* Ehr. from *Hydra* spp., over 70 descriptions of ciliates assigned to the genus have appeared. Recently the morphology and systematics of the genus, its subgenera and species have been reviewed by Lom (1958), and by Uzmann & Stickney (1954).

Fishes, freshwater or marine, are hosts for most species, 53 trichodinid species being so associated. Amphibians are hosts to 12 spp.; molluscs to 10 spp.; echinoderms to 5 spp.; and one species each has been found in or on *Hydra,* turbellarian worms, an echiuroid, and a sponge (Lom, *loc. cit.*; Uzmann & Stickney, *loc. cit.*).

Only three species of *Trichodina* from gastropods have been described, those being *T. patellae* from France (Cuenot, 1893), *T. tegula* from marine turban-shells, (*Tegula* spp.) along the Pacific Coast of California (Hirschfield, 1949), and *T. sphaeronuclea* from the snail, *Schistophallus orientalis,* in Poland (Kazubski, 1958). Four different trichodinids from fresh water snails in California were reported and named by Richards (1949), who failed, however, to describe them other than in his unpublished thesis (1948). Penn (1958) has recently reported trichodinids from freshwater snails in Iowa, but has not described them.

Materials and Methods

In November 1959, several snails, *Helisoma duryi* Say, were taken from the Grove Hall Pond on the campus of the University of Florida in Gainesville. Each had a number of trichodinids in the pulmonary sac. Other snails of the same species taken from nearby ponds on the campus (within a ¼ mile radius) did not have them; nor did apulmonate freshwater snails from the same and other ponds.

I examined the living trichodinids with bright-field, and variable phase-contrast interferometric microscopy at 100X to 1000X;

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and made measurement at dark-phase contrast setting at 400X by means of a calibrated ocular micrometer. A research microscope lamp (set at Köhler adjustment, and equipped with heat absorbent glass, "daylight" blue, ground-glass, and sodium-green glass filters, used singly or in combinations) provided the light.

Twenty-eight organisms from five separate snails were observed in detail while alive; measurements being taken of them. Others were seen but not measured. Four of the 28, in a single group pipetted from the pulmonary sac of a single snail, were fixed in formalin and observed before and after staining. Seven others, in another such group, were anesthetized slowly by .02% $NiSO_4$ solution, so that ciliary placement and numbers were observed in live specimens. The formalin-fixed and anesthetized organisms were also stained with chloroform-extracted methylgreen to extend observations made by interferometric microscopy on the dimensions and positions of nuclei.

Observations

Criteria for specific identification employed by Diller (1928), Fauré-Fremiet (1943), and Dogel (1940), as elaborated by Lom (1958), were used to distinguish the species of the trichodinids.

These criteria when applied to the organisms showed them to be of a new species assignable to the genus *Trichodina*, subgenus *Trichodina* Ehrenberg 1830. Although it closely resembles the organism called *T. helisomarum* by Richards in his unpublished thesis (1948) there are significant differences. It also somewhat resembles the *Trichodina* sp., which Diller (1928) found on tadpoles; and the *T. urinicola f. bohemica* Lom (1958), from newts.

Body shape: The general form is that of a bell, or *cloche* hat, when contracted in the free-swimming stage (Fig. 1); or more broadly bell, or hat-shaped (Figs. 2, 4) if it has only recently released its posterior disc from a holdfast attachment. The body is much flatter when adhering to a substrate.

Size: When flattened against the substrate, the posterior disc of this trichodinid is from 59 to 76 μ in diameter. The majority of those measured were between 68 and 73 μ when the disc was fully flattened and adherent, with a mean of 70 μ and an average of 69.5 μ. The top of the mound of the body mass is 20 to 24 μ above the level of adhesion (Fig. 1). In contracted swimming

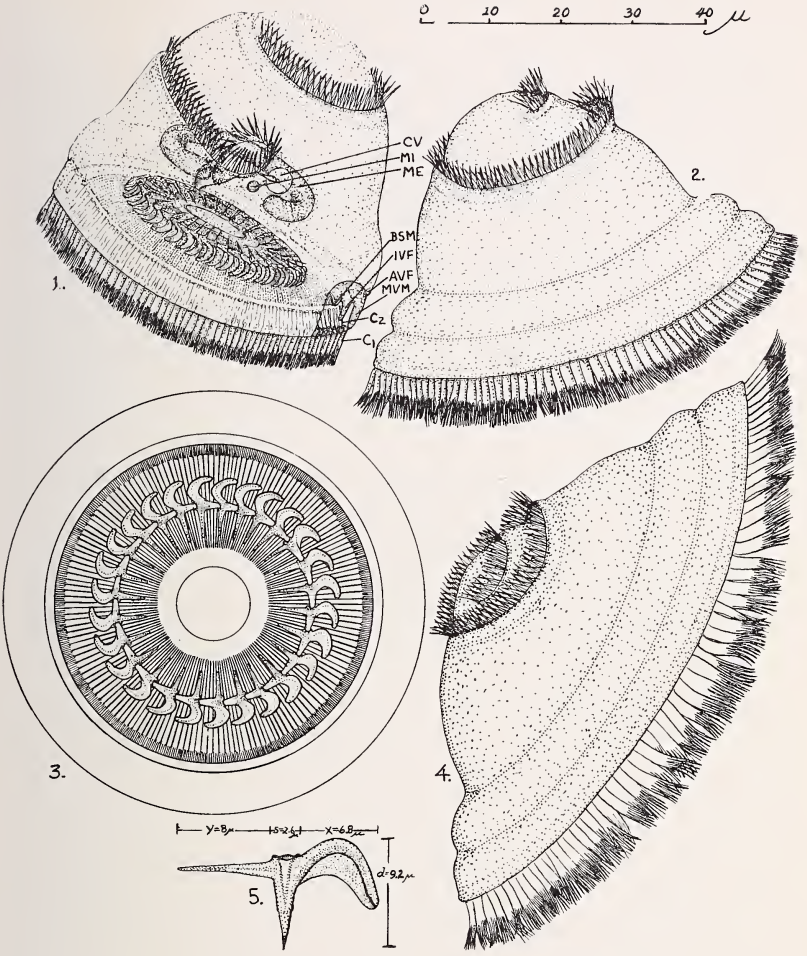

Fig. 1. *Trichodina helisoduria* n. sp. viewed laterally, looking towards the cytopharyngeal vestibule. The meganucleus (ME), micronucleus (MI), and contractile vacuole (CV) with its excretory duct are shown in the vicinity of the vestibule as internal structures. A cut-away at the lower right of the figure shows the border of the striated membrane (BSM), the inner velar fold (IVF), the main velar membrane (MVM), and the accessory velar fold (AVF), the fine cilia (C_2), and the slightly overlapping membranelles (C_1).

Fig. 2. Another lateral view turned 90° to the viewer's right from the previous figure, showing the projecting lip of the cytopharyngeal vestibule.

Fig. 3. The basal adhesive disc, shown flattened and without cilia, depicting the central, clear, circular portion, surrounded by the striated membrane with its denticles, radial pins and fine peripheral striations (which mark the positions of the C_2 cilia), and the borders of the inner and main folds of the velar membrane.

Fig. 4. Another lateral view of the organism showing the flattened condition just after the release of the adhesive disc from its attachment to the substrate.

Fig. 5. Enlargement of a single denticle from the adhesive disc; y = the length of the ray; s = the diameter of the base of the centrum; x = the length of the blade; d = the length of the centrum.

form, the body is 38 to 45 μ high from the edge of the velar border to the adoral surface; less when not contracted, with the diameter across the edges of the velar membrane measuring 45 to 51 μ (Fig. 3). Body diameter just above the adhesive disc is 38 to 42 μ.

Adhesive disc: This organelle consists, when flattened, of (1) a circular central pellicular part 11 to 13 μ in diameter, surrounded by (2) a striated membane 1 to 18 μ diameter (with radial pins 11 to 13 μ long and border membrane, 2 μ in width). This membrane supports (3) a denticulate ring 25 to 28 μ in diameter at the *centra* of the denticles. Peripheral to the border membrane is (4) a velum, composed of an inner fold 1.5 to 2 μ in width, a main velar fold 7 to 8 μ wide, and an external accessory fold, which is scarcely more than a ridge (Fig. 3).

The radial pins of the membrane are about 1 μ in diameter, and are sometimes in pairs, separated by pellicular furrows; but more often are equal in diameter and in length and are unpaired and separated by furrows. There are from 5 to 8 radial pins per denticle, usually 6. The striations of the border membrane are much more numerous, 22 to 25 per denticle, and finer, about 0.4 μ in diameter, barely discernible without staining.

The denticles number from 21 to 32, the majority of organisms having 27 of them. The ray of the denticle[1] is 6 to 9 μ long (Fig. 5), very nearly straight, tapering, with a very slight groove along its length, about 1.2 μ wide at the base adjacent to the centrum, tapering to a slender, slightly rounded tip. The centrum of the denticle is a hollow cone, 2.5 to 3 μ in diameter at the base of the cone,[2] tapering to a pointed tip, with a slight external shoulder about 3.2 μ below the tip of the cone. Its conical, central cavity is about 1.4 μ in diameter at the base, penetrating 4.8 to 5.2 μ into the centrum, regularly tapering. The blade of the denticle has an *x*-length of 6.4 to 7.2 μ, and is broadly sickle-shaped, with a slightly thickened ridge parallelling the posterior curve of the sickle (Fig. 5). Length of the denticle[3] is 8.6 to 10.2 μ.

Ciliary distribution: The *least* conspicuous of the ciliary structures are the delicate cilia which project from between the inner velar fold and the edge of the finely striated basal membrane (Fig. 1), being the "C_2" cilia of Lom (1958). There are several

[1] *u*-portion in Lom (1958).
[2] *s*-breadth.
[3] The *d*-length.

hundred in the circlet. They can be seen clearly by phase-microscopy, and measure 0.3 μ in diameter at the base, tapering to barely resolvable at the tips, being about 4.5 μ long.

The *most* conspicuous of the ciliary structures are the membranelles in a circlet extended from the fundus between the velum and its inner accessory fold and projecting beyond the velum for half, or more, of their lengths. These are composed of six cilia (sometimes five) in a zig-zag row (or perhaps two offset adjacent rows) slightly diagonal to that theoretical radius of the posterior disc which passes through each. Each of these five or six cilia has a separate basal granule. They are adhered for slightly more than half their lengths from the bases, the tips being free. In diameter these cilia are each about 1.2 μ at the base, tapering to less than 0.5 μ at the tip. They vary in length in any one membranelle, 15.5 to 21 μ (Figs. 1, 2, 4).

No marginal cilia anterior to the velum were found, except in one individual which had a few very delicate ones as seen from one side in optical section, anesthetized with $NiSO_4$, and under phase-microscopy. If regularly present in the species they are so delicate as to scarcely discernible.

The cilia of the adoral zone are in two rows of spirally-arranged, heavy, perhaps compound cilia (cirri, or short membranelles) winding counterclockwise along the outer wall of an adoral, spiral groove when observed from above the mouth, ultimately entering the cytopharynx. The two rows, which are offset to one another, complete a 360° turn and a little more before they diverge from one another. The outer row or *haplocinetie,* continues along a slightly extended lip of the cytostome, making nearly 320° of turn before it spirals along the back wall of and into the cytopharyngeal vestibule. Within that it completes the circle, and completes another 360° spiral before ending near the cytostome proper. The inner row, or *polycinetie,* upon divergence from the outer row descends steeply into the cytopharyngeal vestibule, completing 1½ turns, becoming out of phase 180° with the *haplocinetie* but ending near the end of that row close to the cytostome. The cilia in the *haplocinetie* are slightly larger in diameter (1.5 μ) than those of the *polycinetie.* Cilia in each row measure about 6 μ to 8 μ long.

Nuclei: There is one C-shaped meganucleus (macronucleus) just above and almost congruent with the inner circle of the centra

of the denticles. Its outer diameter is 23 to 25 μ; its inner diameter 16 to 17 μ. In length, extended, it would measure 52 to 55 μ, being normally bent into about 6/7 of a circle. The left end of the meganucleus [as seen from the oral pole] lies in the plane of its circle; but the right end is curved adorally out of the plane of the circle, passing just adoral of the cytostome. In cross section the meganucleus is very nearly cylindrical, about 5 μ in diameter. It is less in stained organisms by about 0.75 μ (Fig. 1).

The micronucleus is a dense sphere, 1.8 to 2.1 μ diameter, lying just within the circumference of the circle described by the inner border of the meganucleus; and being 5° to 7° of the circle beyond the left end of the meganucleus; and located just beneath the vestibular lip of the cytopharynx (Fig. 1). It lies in approximately the same plane as the circle of the meganucleus.

Contractile vacuole: This organelle is spherical, vesicle-fed, lying within and just adoral to the arc formed by the right end of the meganucleus. It measures 5-6 μ diameter at beginning of systole; and empties to the cytopharynx by way of a short, permanent duct about 2 μ long and 1.5 μ diameter.

Food vacuoles: There are usually several of these present, clustered around the outer border of the meganucleus, measuring 2.5 to 4.5 μ diameter, containing food in various stages of disintegration. The nature of the food was not determined.

Cytoplasm: The general ground substance of the cytoplasm appears colorless to grayish, and finely granular. No zoochlorellae are present. The course of fibrils in the plasm was not clearly determined, although this could be seen is fixed and stained specimens.

Host specificity: Not clearly determined; but the organism was not found except in the one locale, and then only in the pulmonary sac of *Helisoma duryi*.

Trichodina helisoduria n. sp.

Diagnosis: Inquilinic in pulmonary sac of the freshwater snail, *Helisoma duryi*. Bell to hat-shaped. Diameter of body above adhesive basal disc 38-42 μ. Height of body 38 to 45 μ from edge of velar border of swimming form to adoral crown. Flattened basal disc 59 to 76 μ diameter, average 69.5 μ. Basal disc has central area 11-13 μ diameter; striated membrane 16-18 μ diameter;

border membrane 2 μ wide; supports denticulate ring 25-28 μ diameter across the denticular centra. Peripheral velum has inner fold, main velar fold, and external accessory ridge. Radial pins number 5-8, usually 6, per denticle, about 1 μ diameter, separated by furrows; measure 11-13 μ long. Border membrane finely striated. Denticles 21-32 in number, usually 27; denticular length 8.6-10.2 μ; ray-length, 6-9 μ; centrum, 2.5 to 3 μ diameter; blade length 6.4 to 7.2 μ, ray slightly grooved; blade slightly ridged near posterior curve. Slender delicate ring of cilia from edge of basal membrane, 150 to 200 in number, approximately one per radial pin, 1 μ diameter, 4-5 μ long. Circlet of heavy membranelles between inner and major velar folds, 55-65 in number (about 2 per denticular length), 5-6 cilia per membranelle (1.5 μ diameter; 17-20 μ long) fused near bases. Two rows; adoral cilia; each 1.5 μ diameter at base, 6-8 μ long, over 200 per row, in a spiral of about 405°-410° before entry of either into cytostome. Inner (*polycinetie*) row circles 180° out of phase with outer (*haplocinetie*) row in cytopharynx. Meganucleus 52-55 μ long, 5 μ diameter, bent into 6/7 circle, which is about 20 μ diameter, left end bent slightly adorally. Micronucleus dense, about 2 μ diameter just within circle of meganucleus and 5° to 7° beyond left end of meganucleus. Contractile vacuole single, vesicle fed, empties into cytopharynx.

Discussion

In view of the recent papers by Uzmann & Stickney (1954) and by Lom (1958) in the *Journal of Protozoology* no detailed discussion of the genera and subgenera, species and forms within species, and the characters thereof is warranted here.

Reference is made to those excellent works; and to earlier works of Diller (1928), Dogel (1940), Faurét-Fremiet and his co-workers, Mugard and Thaureux (1924, 1943, 1946) and to other references cited by Uzmann and Stickney (*loc. cit.*) and by Lom (*loc. cit.*).

Table I shows a comparison of the characteristics of *T. helisoduria* n. sp. and those of other species which it resembles [*T. urinicola* f. *bohemica,* and *T. helisomarum*] and of others from which it is quite distinct [*T. myicola* and *T. xenopodis*]. It is plainly similar to *T. helisomarum;* less so to *T. urinicola* f. *bohemica;* definitely distinct from *T. xenopodis*; and still more distinct from *T. myicola.*

The similarity to Richard's (1948) *T. helisomarum* is marked, but there are distinct differences. Richard's organism is larger,

and from a different species of snail (*Helisoma tenue*). The concave margin cited by Richards for *T. helisomarum* is not present in *T. helisoduria* n. sp. The number of denticles in the ring is greater in *T. helisomarum,* and the diameter of the denticulate ring larger; but the blade of the denticle is shorter in *T. helisomarum* than in *T. helisoduria* n. sp. The latter has the fewer radial pins per denticle.

Furthermore, Richards says in his thesis that he found *T. helisomarum* only in *Helisoma tenue*; and he was unable to cross infect other species of snails or other animals (*i.e. Planaria, Hydra*) with *T. helisomarum.* In another, unidentified species of *Helisoma* he found no trichodinids. Short time of survival outside the host is not a factor preventing transfer for *T. helisomarum,* since it lives as long as 14 days free in a hanging drop (Richards, 1948). It may be that it cannot enter or is repelled by, or itself rejects other hosts.

T. heliosoduria n. sp. seems to exhibit a marked host-specificity, also, having been found only in a single species of snail, and in a population of that species from a single pond from an area where that species and other species of snails are abundant in many ponds of similar character.

It seems reasonable that *T. helisoduria* n. sp. is not the same as *T. helisomarum,* even though closely related morphologically, and found in a host snail of the same genus. I believe the evolutionary processes leading to specific differences in the snails may well have resulted in specific differences in these two *Trichodina* spp., both morphologically and in host-specificity.

Summary

1. An inquilinic peritrichous ciliate of the genus *Trichodina* is reported from the pulmonary sac of the freshwater snail, *Helisoma duryi* Say, in Florida.

2. It is compared morphologically to other *Trichodina* spp., and is shown to be similar to *Trichodina helisomarum* reported by Richards (1949), but not described by him in published literature, and similar also to *Trichodina* sp. (Diller, 1928) from tadpoles, and *Trichodina urinicola* f. *bohemica* from newts (Lom, 1958); but different from other *Trichodina* spp.

3. It is described and depicted, and differentiated as a distinct organism, *Trichodina helisoduria* n. sp.

TABLE I

SURVEY OF DATA ON CERTAIN *TRICHODINA* SPP.

Species	Diameter of, in μ			Number of Denticles	Dimensions of Denticle, in μ	Number of Radial Pins per Denticle	Body Shape	Length or Height of Body, μ	Velum	Mega- (macro-) nucleus	Position of Contractile Vacuole	Host
	Body	Adhesive Disc	Denticulate Ring									
T. helisoduria described herein	38–20 40	59–76 69.5	25–28 26.5	21–32 27	d=9.3 s=2.6 y=8.0 x=6.8	5–8 6	Bell-shaped or cloche hat-shaped; discoidal when flat	38–45	Slightly developed	6/7 ring; micronucleus nearly adjacent	Eccentric; empties into cytopharynx	*Helisoma duryi;* a pulmonate snail in freshwater
T. urinicola f. *bohemica* (Lom, 1958)	80	64	42	34	d=7.0 s=3.0 y=7.7 x=7.7	8–10	Half-sphere or bell-like to conical	65	Developed	Horse-shoe shaped; micronucleus adjacent	Eccentric; empties into cytopharynx	*Triturus cristallus;* a newt
T. helisomarum (Richards, 1948)	45–62 52	65–80 *73	25–35 28.5	28–34 31	*d=12.1 s=3.1 y=8.8 x=6.6	7–10 8	Dome-shaped with concave margin	*30–40	Wavy margined; developed	*about 7/8 of a circle with open arc towards cytopharynx; micronucleus adjacent	Eccentric; empties into cytopharynx	*Helisoma tenue; a* pulmonate snail in freshwater
T. xenopodis (Fantham, 1924)	75–92	**	**	48–64	**	**	Vase-like or urn-like	60	**	Horse-shoe shaped with lobes	**	*Xenopus laevis;*
T. myicola (Uzmann and Stickney, 1954)	63–103 81	42–79 62	29–46 36	26–38 29	*d=17.0 s=4.0 y=9.0 x=12.0	5–9	Bell-shaped to discoidal when flat	31–86	Not developed	C-shaped; micronucleus adjacent	Eccentric; empties to body surface	*Mya arenaria;* a marine bi-valve mollusc

* Estimated by measuring author's sketch. ** No data given.

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